Aseptic synthesis of ectomycorrhizae on *Pinus taeda* by basidiospores of *Thelephora terrestris*

DONALD H. MARX AND ELDON W. ROSS
United States Department of Agriculture Forest Service, Southeastern Forest Experiment Station, Athens, Georgia
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Basidiospores of *Thelephora terrestris* introduced into root substrates of aseptic loblolly pine seedlings formed abundant ectomycorrhizae after 2½ months. Cultures isolated from the mycorrhizae were identical with the culture used to form the basidiocarp.

Introduction

Airborne basidiospores are assumed to serve as primary agents of dissemination and inoculum for basidiomycetous ectomycorrhizal fungi. However, only limited evidence supports this contention. Robertson (6) grew *Pinus sylvestris* L. seedlings from surface-sterilized seed in closed and opened bottles of sterile soil in the field. Ectomycorrhizae formed on about 30% of the seedlings in the opened bottles and none developed on seedlings in the closed bottles. Several other workers (1, 7) inoculated various tree seedlings with basidiospores of different symbiotic fungi, but their results were inconclusive. In most instances, noninoculated seedlings formed mycorrhizae from an uncontrolled source of inoculum. Recently, Thapar *et al.* (8) reported synthesis of mycorrhizae on seedlings of *Eucalyptus grandis* (Hill) Maiden with basidiospores of *Scleroderma verrucosum* (Vaill.) Pers. in greenhouse pot culture. Reisolation of the symbiont from the mycorrhizae was not attempted. Noninoculated seedlings did not develop mycorrhizae.

Recent investigations in our laboratory show that *Thelephora terrestris* (Ehrh.) Fr. is symbiotic on roots of several species of *Pinus* (2) and is a pioneer symbiont colonizer of fumigated (3) or autoclaved (4) soil.

Materials and Methods

Basidiocarps of *T. terrestris* (isolate 2) formed in a special plant-growth room (2) were attached with petroleum jelly to lids of sterile, dry, plastic Petri plates and incubated overnight. The basidiocarps were removed, and the plates with numerous basidiospores were stored at 5 °C for 1 month in darkness. Attempts to germinate the basidiospores in darkness at 24 °C were unsuccessful on (*a*) various thiamine- and biotin-supplemented agar media containing different concentrations of glucose and malt extract, (*b*) agar composed of 2% cold-water extract of aseptic loblolly pine feeder roots sterilized by Millipore filtration, and (*c*) detached, aseptic, lateral, and short roots of loblolly pine.

Pinus taeda seeds were surface-sterilized, aseptically germinated, and planted singly in 2-liter jars containing 1 liter of sterilized vermiculite–peat moss–nutrient substrate at pH 5.5 (5). After 3 months' growth in a greenhouse water bath (20 °C), 10 ml of an aqueous suspension of basidiospores (about 10 000 spores) was introduced to root substrates of 16 seedlings and sterile water was added to 6 control seedlings.

Results

One month after inoculation, white mycelial strands typical of *T. terrestris* (4) were observed in the root substrate of about half the inoculated seedlings. Two weeks later, bifurcate mycorrhizae were observed on lateral roots next to the walls of the glass jars. Ten weeks after inoculation, the seedlings were removed from the jars and their roots were examined. Six inoculated seedlings were heavily contaminated with a species of *Penicillium* and did not have mycorrhizae. The other 10 inoculated seedlings had abundant mycorrhizae. The noninoculated seedlings did not have mycorrhizae.

Several lateral root segments (2–3 cm long) with mycorrhizae were surface-sterilized in aqueous mercuric chloride (100 parts per million) for ½ to 1½ minutes. After a thorough rinsing in sterile water, 30 mycorrhizae from each mycorrhizal seedling were excised from the root

segments, placed separately in tubes of modified Melin-Norkrans agar medium (3), and incubated at 24 °C for 6 weeks. *Thelephora terrestris* was reisolated from 3 to 27% of the mycorrhizae of each mycorrhizal seedling. The reisolated cultures were culturally and microscopically identical with the parent culture (3). Histological examination of the mycorrhizae revealed that the Hartig net, fungus mantle, and type of hyphal clamp connections were identical with those previously described for this symbiotic association (4).

Discussion

We conclude that basidiospores of *T. terrestris* function as inoculum for mycorrhizal synthesis and, in concurrence with recent work (2, 3, 4), that they also serve as agents of airborne dissemination. Proof of the function of basidiospores of hymenomycetous symbiotic fungi is essential to our understanding of the ecology of mycorrhizal associations, especially in respect to reforestation problems and the colonization of soils that have been fumigated or are otherwise free of symbionts.

The germination of basidiospores in the root substrate of aseptic pine seedlings and the lack of germination on various agar media, root extracts, and detached pine feeder roots strongly suggests that living roots are essential for germination of basidiospores of *T. terrestris*.

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